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MICROFLORA OF ALLERGENIC POLLENS - A PRELIMINARY STUDY*

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Abstract: Eight samples of allergenic pollen originating from five species of anemophilous plants were investigated in order to estimate their contamination with mesophilic bacteria, thermophilic actinomycetes and fungi. The concentrations of Gram-positive bacteria ranged from 0 to 21000 cfu/g, of Gram-negative bacteria - 0 to 30000 cfu/g, of thermophilic actinomycetes - 0 to 10000 cfu/g, and concentration of fungi ranged from 0 to 34000 cfu/g. It is suggested that further study of the microflora of allergenic pollens and its potential role in pollen allergy is needed.

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INTRODUCTION

Although numerous studies of microflora of plant surfaces have been carried out, until now very little attention has been paid to microorganisms and their spores present on pollen. In a previous study [17] the presence of Gram-negative bacteria and endotoxin on pollen surfaces has been shown. Endotoxin is a known airborne immunotoxicant for humans and animals [6, 7, 13, 14]. Nevertheless, the knowledge of bacteria and fungi associated with pollen is incomplete. Accordingly, the aim of this study was to perform a preliminary examination of the microflora present on pollen.

MATERIALS AND METHODS

Pollen. Eight pollen samples of five plant species were examined in 1995 to determine concentrations and species composition of bacteria and fungi. The samples were collected during flowering seasons 1994 and 1995 from the following plants: 1) rye (*Secale cereale*) in 1994; 2) mugwort *(Artemisia vulgaris)* in 1994; 3) hazel *(Corylus avellana)* in 1995; 4) European alder (*Alnus glutinosa*) in 1995 - all from ecologically clean regions in Kraków Province (Southern Poland). Additionally, white warty birch *(Betula verrucosa)* pollen was collected four times - 5) in 1994 (lot # 1) and 6) in 1995 again (lot # 2) from an ecologically clean region in Kraków Province, 7) in 1995 (lot #3) from a housing estate located in the City of Kraków, and 8) in 1995 (lot #4) from a polluted industrial area near a cokery in Kraków. The following technique of pollen sampling was used: the flower-bearing stems (branches) were cut before starting pollination and then placed in flagons into sterile chambers and allowed to pollinate. The falling pollen was collected onto a sterilised foil and then kept deep-frozen until the examination.

The concentration of bacteria in the pollen samples was determined by dilution plating. One hundred mg of each sample was suspended in 10 ml of sterile 0.9% NaCl solution containing 0.1% (v/v) of Tween 80. After vigorous shaking, 10-fold serial dilutions were made up to 10^{-4}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of media appropriate for determination of mesophilic bacteria, thermophilic actinomycetes and fungi. The data were reported as colony forming units (cfu) per 1 g of pollen.

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Table 1. Numbers of mesophilic bacteria on allergenic pollens

Pollen sample	Harvesting year	Place of pollen collecting	Gram-positive bacteria (cfu/g)	Gram-negative bacteria (cfu/g)	Total
Rye (*Secale cereale*)	1994	Ecologically clean region	2000	1000	3000
Mugwort (*Artemisia vulgaris)*	1994	Ecologically clean region	21000	7000	28000
Hazel (*Corylus avellana*)	1995	Ecologically clean region	0	2000	2000
Alder (*Alnus glutinosa*)	1995	Ecologically clean region	11000	0	11000
Birch (*Betula verrucosa*) - lot # 1	1994	Ecologically clean region	0	30000	30000
Birch (*Betula verrucosa*) - lot # 2	1995	Ecologically clean region	8000	0	8000
Birch (*Betula verrucosa*) - lot # 3	1995	Housing estate	2000	28500	30500
Birch (*Betula verrucosa*) - lot # 4	1995	Neighbourhood of a cokery	0	0	0

Determination of total mesophilic bacteria. The serial dilutions of sample Nos 1–8 were inoculated on blood agar. The plates were incubated for one day at 37°C, for the next three days at 22°C, and finally for three days at 4°C [10]. Colonies were counted and differentiated on the basis of morphology and Gram staining. Isolates were determined according to the Bergey's Manual [3, 4].

Determination of thermophilic actinomycetes. The dilutions of sample Nos 1–4 were inoculated on half-strength tryptic soya agar for thermophilic actinomycetes. The plates were incubated for five days at 55°C. Colonies were counted and isolates were determined to the level of genus or species according to the Bergey's Manual [5].

Determination of fungi. The serial dilutions of sample Nos 1–4 were cultured on malt agar (Difco). The plates were incubated for four days at 30°C, and for the next four days at 22°C. The colonies were counted and fungi were determined using the manuals of Baron [2] and Litvinov [12].

RESULTS

Table 1 presents numbers of mesophilic bacteria in examined pollen. It may be seen that the numbers of Gram-positive bacteria ranged from 0–21000 cfu/g, while those of Gram-negative bacteria were between 0–30000 cfu/g.

Thermophilic actinomycetes were less numerous than mesophilic bacteria (Tab. 2) and their numbers ranged from 0–10000 cfu/g pollen. The dominant species was *Thermoactinomyces thalpophilus*.

Table 3 presents the numbers of fungi in four samples of allergenic pollen. The concentration of fungi ranged from 0–47000 cfu/g. The prevailing fungi were *Cladosporium herbarum* (total 34000 cfu/g), followed by yeasts (11000 cfu/g), and *Alternaria alternata* (10500 cfu/g).

DISCUSSION

Although the influence of environmental factors upon the etiology and course of allergic rhinits has been widely discussed [15], so far little attention has been paid to microflora associated with the pollen grains. Colldahl and Carlsson [8] cultured from pollen samples fungus *Cryptococcus luteolus* and a Gram-negative bacterium *Pseudomonas maltophilia* as typical microorganisms associated with pollen. The presence of microorganisms on surface of pollen grain was subsequently confirmed by means of scanning electron microscopy [9]. Śpiewak *et al.* [17] showed that Gram-negative bacteria *Pantoea agglomerans* (synonyms: *Erwinia herbicola, Enterobacter agglomerans*) are present on pollen grains as well as endotoxin - the bacterial product having strong immunomodulating properties. The present study confirms the previous results by use of another culture medium, revealing the

Table 2. Numbers of thermophilic actinomycetes on allergenic pollens

Pollen sample	Harvesting year	Place of pollen collecting	*Thermoactinomyces vulgaris*	*Thermoactinomyces thalpophilus*	*Streptomyces* spp.	Total
Rye (*Secale cereale*)	1994	Ecologically clean region	0	0	0	0
Mugwort (*Artemisia vulgaris)*	1994	Ecologically clean region	2500	1000	5000	8500
Alder (*Alnus glutinosa*)	1995	Ecologically clean region	0	10000	0	10000
Birch (*Betula verrucosa*) - lot # 1	1994	Ecologically clean region	0	10000	0	10000

Table 3. Numbers of fungi on allergenic pollens

Pollen sample	Harvesting year	Place of pollen collecting	*Alternaria alternata*	*Oidiodendron flavum*	*Cephalosporium charticola*	*Cladosporium herbarum*	*Penicillium* sp.	Yeasts	Total
Rye (*Secale cereale*)	1994	Ecologically clean region	0	0	0	0	0	0	0
Mugwort (*Artemisia vulgaris)*	1994	Ecologically clean region	10500	4500	1000	0	2000	0	18000
Alder (*Alnus glutinosa)*	1995	Ecologically clean region	0	0	0	0	2500	0	2500
Birch (*Betula verrucosa*) - lot # 1	1994	Ecologically clean region	0	0	0	34000	2000	11000	47000

presence of Gram-negative bacteria on the surface of allergenic pollens, as well as the presence of Gram-positive bacteria, thermophilic actinomycetes and different species of fungi. So far, it is not clear how the pollens were contaminated by these microorganisms. It could have happened before leaving the pollen sac, but more likely the contamination may have occured during falling of pollen. Although in our experiment the surrounding of pollinating plants was sterilised, the collected pollen could have been contaminated by microorganisms falling from the unsterilised plant surfaces. In nature, pollen could probably be contaminated also by interaction with other bioaerosols. In our experiment, however, this way does not seem possible as the pollinating process was completed in a previously sterilised chamber, and there were no considerable air movements after placing the stems or branches into the chambers. Thus, further studies are needed in order to determine the way of microbial contamination of pollen in natural conditions.

It cannot be excluded that a part of allergic symptoms caused by exposure to pollens may be due to the presence of microorganisms and their products on pollen grains. Though the concentrations of microorganisms in pollen were much smaller compared to those reported from various organic dusts [10, 11], they are able to evoke some response after inhalation. It is known that even small quantities of bacterial and fungal products may cause various adverse effects which can be attributed to allergic or immunotoxic reactions [7, 13]. For example, it has been shown that endotoxin produced by Gram-negative bacteria activates macrophages to produce IL-1, TNF and other cytokines [1, 6, 7] initiating inflammatory processes in the airways. *Thermoactinomyces* produces strong allergens and is one of the major agents causing allergic alveolitis [6, 11]. Fungi and their products, similarly to bacteria, can cause allergic and immunotoxic reactions. For example, fungi belonging to the genera *Penicillium, Alternaria and Cladosporium* possess strong allergenic properties [11, 16]. However, on the basis of the hitherto obtained data it is difficult to say whether the presence of the particular fungal spores or mycelia plays a relevant role in the development of symptoms in patients sensitive to pollens.

CONCLUSION

A mixed microflora, consisting of Gram-positive and Gram-negative mesophilic bacteria, thermophilic actinomycetes and fungi, is present on allergenic pollens. Further studies are needed to determine their potential role in the etiopathogenesis of pollinosis.

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